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BY

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The authors have recently described a method (1) for the determination of the sugars of honey in which the sample is first subjected to carbon column chromatography to separate the sugars into monosaccharide, disaccharide, and higher sugar fractions. In the present paper are given results of the application of this method to 21 honey samples, representing 19 floral types of honey.

Many polarimetric and reduction methods have been used for the analysis of honey. These methods have not lacked in precision (2), but no assurance of accuracy can be obtained from an examination of the literature. For routine analytical purposes, honey has been regarded as a mixture of glucose and fructose with small amounts of sucrose, and of ill-defined carbohydrate materials collectively analyzed as honey "dextrin." Maltose, identified as the osazone (3), has been reported to occur in honey. van Voorst has described the application of his differential fermentation to 41 honey samples and stated that all contained maltose in amounts ranging from 2 to 7 per cent (4). Hurd, Englis, Bonner, and Rogers later applied a distillation method of sugar analysis to several honey samples. They found "maltose or some other reducing disaccharide" (5) to be present in all five samples examined. Neither of these methods is particularly well adapted to routine analysis, although that of van Voorst is somewhat better than that of Hurd, *et al.*

Paper chromatography shows promise of being most valuable in application to the problem of the identity of the sugars of honey. It has been used for this purpose by Täufel and Reiss (6), Malyoth (7), Vavruch (8), and at this laboratory. In general, no sugars have unequivocally been added to the list occurring in honey, but the complexity of the mixture in indicated. Täufel and Reiss have reported a total of 9 sugars, with 5 unidentified.

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METHODS OF ANALYSIS

The detailed method of analysis is described in another paper (1). In principle, the honey sample (0.8–1.0 g.) is subjected to adsorption on a column of carbon–celite under controlled conditions. Three fractions are obtained by successive elution:

(*A*) Monosaccharides in 1 per cent ethanol.
(*B*) Disaccharides in 7 per cent ethanol.
(*C*) Higher sugars in 50 per cent ethanol.

Glucose and fructose are determined in the *A* fraction by modification (1) of the Marshall–Norman (9) procedure. Sucrose in the *B* fraction is estimated by increase in reducing power following mild acid hydrolysis. Reducing disaccharides in the *B* fraction are determined by copper reduction calibrated against maltose. The higher sugars in the *C* fraction are determined by the reducing power after hydrolysis and are reported as glucose.

Replication.—For each honey type, a single sample was subjected to adsorption. For fructose, duplicate reducing-sugar determinations were done on aliquots from a single hypoiodite oxidation. For glucose, duplicate hypoiodite oxidations were done. For sucrose, duplicate aliquots from the 7 per cent ethanol eluate were hydrolyzed and one reducing sugar determination was done on each. For maltose, duplicate reducing sugar determinations were done on aliquots of the *B* fraction. For the *C* fraction, a single strong acid hydrolysis was done and duplicate reducing sugar values were obtained. In general, Shaffer–Somogyi titers agreed within ± 0.07 ml of .005 *N* thiosulfate; dextrose titers within ± 0.05 ml. of .05 *N* thiosulfate. The sucrose values are those found analytically, corrected for the 94.4 per cent recovery previously determined for the carbon column (1). Maltose values are likewise corrected for the 98.4 per cent recovery found for the columns. Other values are as found.

Paper chromatography of fractions.—As a routine check on the completeness of the carbon column separations, each fraction from the columns for each honey sample was examined by paper chromatography. The chromogenic reagent used (benzidine–citric acid (10)) is sensitive to 1 microgram of sugar on the paper. No appreciable contamination by components of other fractions was found. Traces of sugars from adjoining fractions were shown in the *B* and *C* fractions, but the authors believe that these are analytically insignificant. McDonald and Perry, using ammoniacal silver nitrate in a similar check of their carbon-column analysis (11) of corn sirups, state that no overlapping of the fractions was found. This may be due to the lower sensitivity of the latter reagent.

Figure 1 shows a typical papergram of the three fractions. It is obvious that the *B* fraction (disaccharides) is not limited to maltose and sucrose and that the *C* fraction (trisaccharides and higher sugars) is also a complex mixture. The monosaccharide fractions were all found to contain glucose

and fructose alone with no contamination by other sugars. This indicates that inaccuracy in the determination of these two sugars in honey arising from the presence of other sugars is eliminated and that the glucose and fructose values obtained by this procedure are closer to the actual composition of the honey than those obtained by other methods.

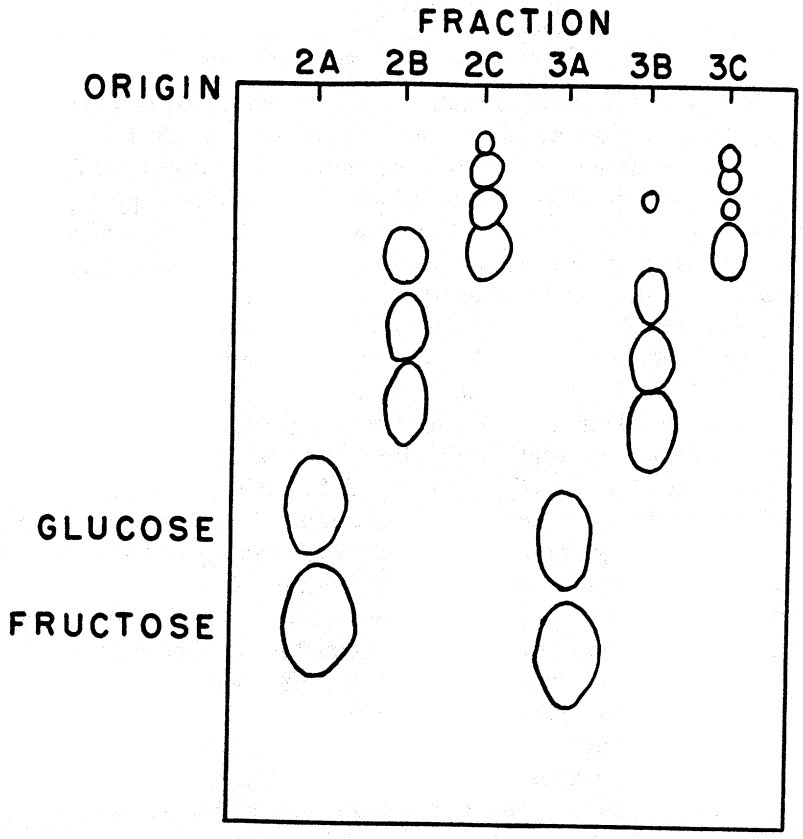

FIG. 1.—Tracing of paper chromatogram of carbon column fractions for samples 2 and 3. Downward irrigation with *n*-butanol–pyridine–water, 3:1:1.5; color spray benzidine–citric acid. The spots of identical R_F in fractions 3B and 3C have been differentiated by other color reagents.

No claim is made that the reducing disaccharides determined in honey are in fact pure maltose, but for convenience they are so calculated. Figure 1 shows several sugars, three of which are reducing.

The *C*, or higher sugar fraction, probably contains the components determined by conventional honey analyses as "dextrins." It is apparent from a comparison of Figure 1 with the photograph of Buchan and Savage's papergrams of starch conversion products (12) that they are

not true dextrins. The authors believe that the term "higher sugars" is more appropriate for the carbohydrates of fraction *C* from honey.

RESULTS AND DISCUSSION

In Table 1 are shown the results of the application of this analytical procedure to 22 samples (21 honey and 1 honeydew) from 20 different

TABLE 1.—*Carbohydrate composition of honey as determined by selective adsorption method*

NO.	FLORAL SOURCE	CROP YEAR	H_2O[a]	GLUCOSE	FRUCTOSE	MALTOSE	SUCROSE	HIGHER SUGARS	TOTAL SUGARS
			per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	Spanish Needle	1949	17.80	23.52	38.38	14.02	1.11	1.43	78.46
2	Calif. Sage	1948	14.00	25.78	41.97	11.66	1.49	2.54	83.44
3	Tupelo	1951	15.88	24.16	42.42	10.17	1.42	2.50	80.67
4	Basswood	1951	18.04	33.04	37.06	6.84	1.51	2.13	80.58
5	Heartsease	1951	19.08	28.04	38.84	9.16	0.33	0.78	77.15
6	White Thistle	1951	15.84	31.60	36.26	7.58	3.57	3.30	82.31
7	Fallflower	1951	18.00	33.82	37.86	6.16	1.15	0.80	79.79
8	Mesquite	1952	17.64	36.83	38.49	4.34	1.24	0.16	81.06
9	Alfalfa (Calif.)	1951	16.12	34.50	37.29	7.28	4.27	0.68	84.02
10	Eucalyptus	1951	16.60	32.98	39.81	7.80	1.04	0.74	82.37
11	Sweet Clover	1951	16.20	34.36	38.33	6.04	2.57	0.87	82.17
12	Alfalfa (Ariz.)	1951	16.20	33.76	39.13	6.46	1.19	0.65	81.19
13	Calif. Buckwheat	1951	13.40	32.20	41.42	8.15	0.46	0.67	82.90
14	Cotton	1952	19.36	33.66	37.80	6.00	1.11	0.46	79.03
15	Calif. Orange	1952	16.40	33.86	38.88	6.26	3.03	1.16	83.19
16	White Clover	1951	18.12	33.50	37.85	6.33	1.59	0.98	80.25
17	Buckwheat	1951	17.64	34.36	38.67	5.79	0.73	0.80	80.35
18	Pine Tree Forest[b]	1950	14.96	22.18	32.62	18.13	0.96	6.96	80.85
19	Wild Thyme	1950	14.60	24.87	42.42	10.17	1.50	2.50	81.46
20	Dandelion	1952	16.48	40.95	35.53	4.48	1.06	0.47	82.49
21	Tupelo	1953	16.60	26.54	43.95	6.38	0.52	1.74	79.13
22	Gallberry	1953	15.32	30.78	40.52	7.45	1.50	1.18	81.43
	Average		16.72	32.29	39.28	7.11	1.62	1.03	81.31

[a] By refractometer.
Honeydew not included in average.

sources. Table 2 shows the analyses of the first 19 of these samples by other analytical methods. Samples 2, 18, and 19 were analyzed by the A.O.A.C. polarimetric method (13); the others by the Lothrop–Holmes (14) method. Sucrose was determined in all samples by the A.O.A.C. acid hydrolysis procedure (13). These analyses were carried out about six months previous to those in Table 1. In general, the new method gives lower glucose values than previous ones and shows the presence in all samples of reducing disaccharides, which were calculated as maltose. This confirms the observations of van Voorst; good agreement is also shown with the values reported by Hurd, *et al.*, in which the sugars were also separated into mono-, di-, and higher saccharides by distillation of the propionates.

TABLE 2.—*Carbohydrate analysis of honey (per cent) by conventional methods*[a]

NO.	H_2O[b]	GLUCOSE	FRUCTOSE	SUCROSE[c]
1	18.12	32.3	41.5	3.6
2	14.68	31.2	45.3	2.5
3	15.88	28.2	43.8	2.4
4	18.12	35.9	37.0	2.3
5	19.40	33.2	37.2	1.2
6	15.88	33.7	39.6	5.0
7	18.00	37.5	37.4	1.6
8	17.80	39.3	38.9	1.8
9	15.60	37.5	39.0	4.7
10	16.60	36.2	41.6	1.5
11	16.16	37.0	41.0	3.0
12	16.16	37.0	41.8	1.5
13	13.40	36.3	42.3	0.9
14	19.36	37.0	38.8	1.6
15	16.56	35.9	39.6	4.0
16	18.64	36.5	38.1	2.6
17	17.48	38.8	39.0	1.7
18	16.52	28.0	34.0	8.15
19	15.32	31.2	45.4	1.98

[a] Samples 2, 18, 19, by A.O.A.C. polarimetric method; 1, 3–17, by Lothrop-Holmes method.
[b] By refractometer.
[c] Authors are indebted to Mrs. M. S. Gaspar for these sucrose analyses.

Table 3 shows a comparison of the values for selected samples from Table 1 with analyses reported by Hurd, *et al.* (5) for honey samples from the same floral sources. These latter were determined by conversion of the sugars to proprionate esters, followed by fractional distillation; the "tri- and higher saccharides" were calculated from the residue after distillation. Hurd's values as shown in Table 3 were recalculated to the same moisture contents as the samples in this laboratory to facilitate comparison. Agreement is reasonably good, especially in the disaccharide fractions.

The average analyses of honey as reported by several investigators are shown in Table 4. Also shown in Table 4 is the average of the 19 samples reported in Table 2, analyzed by conventional procedures, and the average of the same 19 samples analyzed by the new procedure.

Glucose.—The average content of this sugar was lower by 4 per cent (11.5 per cent less on the individual sugar basis) when determined by the new method than when the same samples were analyzed by other procedures. The average glucose content of all domestic samples in Table 1 is about 2 per cent lower than reported for the 198 samples of Browne and of Eckert and Allinger.

Fructose.—The average fructose content is somewhat lower (1.35 per cent, or 3.3 per cent on the fructose basis) when analyzed by the new method than was found by other methods on the same samples. It is 1.65

TABLE 3.—*Comparison of honey analyses by carbon column adsorption (A) with those reported for a distillation method (B)*

NO.	FLORAL SOURCE	METHOD	H_2O	MONO-SACCHARIDE	DI-SACCHARIDE	TRI- AND HIGHER SACCHARIDE
			per cent	*per cent*	*per cent*	*per cent*
11	Sweet Clover	A	16.2	72.7	8.6	0.9
		B	16.2[a]	74.8	6.3	2.5
5	Heartsease	A	19.1	66.9	9.5	0.8
		B	19.1[a]	66.4	10.1	3.8
15	Calif. Orange	A	16.4	72.7	9.3	1.2
		B	16.4[a]	70.1	10.8	2.7
17	Buckwheat	A	17.6	73.0	6.5	0.8
		B	17.6[a]	70.6	6.9	1.9
3	Tupelo	A	15.9	66.6	11.6	2.5
		B	15.9[a]	68.6	11.4	4.1

[a] All values shown for method B have been recalculated from the original data (Table 1 in ref. (5)) to the moisture contents shown for method A.

per cent lower than the average value for the 198 samples referred to above. This discrepancy may in part be brought about by the presence of reducing ketose (fructose) disaccharides and higher sugars determined as fructose in older methods. This may be confirmed by the determination of reducing ketose groups in the disaccharide fraction. In one honey reported in Table 1 (No. 16) such a determination gave a value of 0.9 per cent for reducing ketose disaccharide content, equivalent in reducing power to 0.5 per cent fructose.

Sucrose.—The average sucrose content by the adsorption method is also lower than was found for the same samples by conventional methods

TABLE 4.—*Average composition of honey as determined by different methods*

SAMPLES	H_2O	GLUCOSE	FRUCTOSE	SUCROSE	MALTOSE	DEXTRIN	METHODS
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	
19 Domestic	16.75	32.20	38.80	1.59	7.47	1.24	Selective adsorption, this paper[a]
19 from Table 2	16.82	34.9	40.1	2.7			cf. Footnote a, Table 2
19 from Table 1	16.63	30.90	38.75	1.58	8.33	1.62	Selective adsorption, this paper[b]
92 Domestic	17.70	34.02	40.50	1.90		1.51	Polarimetric; reduction (18)
106 California	16.04	34.54	40.41	2.53		0.91	A.O.A.C.: polarimetric, reduction (19)
41 European		30.5	41.5		4.6	1.7	Reduction, differential fermentation (4)

[a] Table 1 with samples 18, 19, 21 excluded.
[b] Table 1, with samples 20, 21, 22 excluded, hence the same 19 samples reported in Table 2.

TABLE 5.—*Approximate melezitose content of honeys and honeydews*

NO.	SOURCE	MELEZITOSE	HIGHER SUGARS
		per cent	*per cent*
18	Pine-tree honeydew	4.33	6.96
19	Wild thyme	0.18	2.50
23	Honeydew mixture	3.51	7.50
24	Honeydew mixture	2.91	6.72
25	Honeydew mixture	3.96	6.31
6	White thistle	1.90	3.30

(Table 4). In both cases the acid hydrolysis procedure was used. It is probable that this difference is caused by sucrose-type linkages in tri- or higher saccharides in the *C* fraction. Such sugars as melezitose or maltosyl fructose (15) would simulate sucrose in the conventional analytical procedure. For example, sample 18 showed 8.15 per cent sucrose by the old procedure and only 0.96 by the new. As shown in Table 5, this honey contains 4.33 per cent of higher sugars containing labile linkages, calculated as melezitose. Sample 19, in which sucrose by both procedures is reasonably in agreement, showed (Table 5) only 0.18 per cent of such compounds.

Maltose.—The presence of reducing disaccharides in the honey samples reported in Table 2 is ignored in the analytical procedures used. As seen in Table 4, these same samples averaged 8.33 per cent of reducing disaccharides (calculated as maltose). This amount of disaccharide would simulate $180/342 \times 8.33 = 4.38$ per cent glucose if determined by A.O.A.C. methods. This value may be compared with the average 4.0 per cent lower glucose content for these samples found by the selective adsorption procedure.

Dextrin.—No comparative values are available for dextrin by conventional procedure and higher sugars by the new method on the same samples. However, the 1.30 per cent average for higher sugars for the 22 samples in Table 1 may be compared with the average of 1.19 per cent dextrin for the 198 samples analyzed by Browne and by Eckert and Al Allinger (Table 4). These 198 samples were analyzed for "dextrin" by alcoholic precipitation, while the new method determines higher sugars after isolation from monosaccharides and disaccharides, by copper reduction after hydrolysis.

Determination of melezitose.—The trisaccharide melezitose is a component of honeydew (16). The melezitose content of a sample can be approximated from the increase of reducing power brought about by the mild hydrolysis of the "*C*" fraction. According to von Fellenberg (17), the application of mild hydrolysis to melezitose produces reducing sugar equivalent to 67.9 per cent of its weight of glucose. The higher sugar fraction of several samples has been analyzed in this manner. Results are shown in Table 5.

Undetermined.—It may be seen from Table 1 that on the average 1.95 per cent of the 21 honey samples remains unaccounted for. This undetermined fraction varies from 4.27 per cent in sample 21 (tupelo) to zero (within the limits of error of the analysis) for samples 9 (California alfalfa) and 15 (California orange). Browne (18) reported 3.7 per cent undetermined for his 92 domestic samples; Eckert and Allinger (19) reported 4.7 per cent. For the 25 samples reported by Lynn, *et al.* (20), 4.1 per cent was not accounted for. The principal reason for this improvement over older procedures is the reducing disaccharide fraction, representing an average of 7.11 per cent. In the A.O.A.C. procedure, it is analyzed on the basis of its reducing value and simulates about half its weight of glucose. Thus there is a net gain in dry matter recovery.

A picture of the carbohydrate composition of honey may be deduced from these considerations. Although based upon only 21 samples of 19 different floral types, the following generalizations are proposed. Honey contains somewhat less dextrose and levulose than heretofore supposed, less sucrose, and appreciable amounts of reducing disaccharide material which is largely aldose, some reducing ketose disaccharide, and small amounts of trisaccharide and of higher sugars, some of which contain linkages of about the same ease of hydrolysis as the sucrose linkage.

SUMMARY

Twenty-one honey samples representing 19 floral sources have been analyzed for carbohydrate content by a new selective adsorption procedure. Average values found were: moisture, 16.72 per cent; glucose, 32.29 per cent; fructose, 39.28 per cent; sucrose, 1.62 per cent; maltose, 7.11 per cent; and higher sugars, 1.03 per cent. This represents a considerably lower glucose content, somewhat lower fructose and sucrose levels, and significant amounts of reducing disaccharides (as maltose) when compared with average results by previous methods. Previously postulated occurrence of reducing disaccharides in honey is confirmed.

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REFERENCES

(1) White, J. W., Jr., and Maher, J., *This Journal*, **37**, 466 (1954).
(2) White, J. W., Jr., Ricciuti, C., and Maher, J., *ibid.*, **35**, 859 (1952).
(3) Elser, E., *Mitt. Gebiete Lebensm. Hyg.*, **15**, 92 (1924).
(4) Voorst, F. T. v., *Z. Untersuch. Lebensm.*, **83**, 414 (1942).

(5) HURD, C. D., ENGLIS, D. T., BONNER, W. A., and ROGERS, M. A., *J. Am. Chem. Soc.*, **66**, 2015 (1944).
(6) TÄUFEL, K., and REISS, R., *Z. Lebensm.-Untersuch. u. -Forsch.*, **94**, 1, 1952.
(7) MALYOTH, E., *Naturwiss.*, **38**, 478 (1951).
(8) VAVRUCH, I., *Chem. Listy*, **46**, 116 (1952); through *Chem. Abst.*, **46**, 11497h (1952).
(9) MARSHALL, C. R., and NORMAN, A. G., *Analyst*, **63**, 315 (1938).
(10) WHITE, J. W., JR., and MAHER, J., *Arch. Biochem. Biophys.*, **42**, 360 (1953).
(11) MCDONALD, E. J., and PERRY, R. E., JR., *J. Research, Natl. Bur. Standards*, **47**, 363 (1951).
(12) BUCHAN, J. L., and SAVAGE, R. I., *Analyst*, **77**, 401 (1952).
(13) *Official Methods of Analysis*, 7th Ed., Association of Official Agricultural Chemists, Box 540, Benjamin Franklin Station, Washington 4, D. C., 1950.
(14) LOTHROP, R. E., and HOLMES, R. L., *Ind. Eng. Chem., Anal. Ed.*, **3**, 334 (1931).
(15) WHITE, J. W., JR., and MAHER, J., *J. Am. Chem. Soc.*, **75**, 1259 (1953).
(16) HUDSON, C. S., and SHERWOOD, S. F., *ibid.*, **42**, 116 (1920).
(17) FELLENBERG, T. v., and RUFFY, J., *Mitt. Gebiete Lebensm. Hyg.*, **24**, 367 (1933).
(18) BROWNE, C. A., *Bull. 110, Bur. Chem.*, U. S. Department of Agriculture, 1908.
(19) ECKERT, J. E., and ALLINGER, H. W., *Bull. 631, Agric. Expt. Sta.*, University of California (1939).
(20) LYNN, G. E., MILUM, V. G., and ENGLIS, D. T., *33rd and 34th Ann. Rept., Ill. State Beekeeper's Assoc.*, 54 (1934).